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Risk factor of Candida morphology change and colonization increase in critically ill patient in ICU

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Abstract— Invasive candidiasis is the most commonly encountered fungal disease in intensive care units. Year after year, the mortality rate rises for a variety of reasons. Fungus colonization has drawn attention and been acknowledged as a separate risk factor for the emergence of invasive candidiasis.This has been demonstrated in numerous investigations using Candida colonization as a grading system and a tool for predicting the emergence of invasive candidiasis. This study is a prospective observational cohort study to examine the relationship between risk factors and Candida morphology change and colonization increase in axilla and rectal swabs in critically ill patient while considering time exposure of each factor.Inclusion criteria were ICU patients aged 18-70 years old who were predicted to stay more than two days, sequential organ failure assessment (SOFA) scores more than two.Parenteral nutrition, leukocyte day 1, old age and female have a hazard ratio more than one, which it means all risk was associated with Candida morphology change and colonization increase in axilla and rectal swabs. Further research is needed to determine the likelihood Candida morphology change and colonization increase in axilla and rectal swabs, such as system scoring. It will help clinician to know how to start antifungal administration.

Keywords: Candida, morphology, colonization, critically ill

1. Introduction

Invasive candidiasis is the most commonly encountered fungal disease in intensive care units.[1] Year after year, the mortality rate rises for a variety of reasons, including a lack of feasible, rapid, and accurate diagnostic methods, which often leads to delayed introduction of antifungal medications and is subsequently linked to a worse prognosis, especially given that blood culture results often return late in the course of infection.[2-4]

In several articles, fungus colonization has drawn attention and been acknowledged as a separate risk factor

for the emergence of invasive candidiasis. Colonization precedes invasive infections, hence the presence of colonization is considered to be potentially indicative of invasive candidiasis development. This has been demonstrated in numerous investigations using Candida colonization as a grading system and a tool for predicting the emergence of invasive candidiasis.[1,2,5]

By examining candida colonization, this study aimed to lower the incidence and mortality from invasive candidiasis. It was also the objective of this study to identify risk factors in Candida morphology changes and colonization increase in axilla and rectal swabs in critically ill patients in ICU while considering time exposure of each factor.

2. Methods

2.1 Study setting and protocol

This study is a prospective observational cohort study to examine the relationship between risk factors and Candida morphology change and colonization increase in axilla and rectal swabs in critically ill patient. Inclusion criteria were ICU patients aged 18-70 years old who were predicted to stay more than two days, sequential organ failure assessment (SOFA) scores more than two. Exclusion criteria were post-cardiac surgery, pregnancy, immunocompromise, malignancy, and patients who declared themselves out of the study. Subject with positive colonization in first examination was excluded too. Swabs were obtained on the first day patient admitted in ICU. Subjects were observed during the nine days of the study and samples were taken from blood, skin swabs, and rectal swabs on 1st, 5th, and 9th day for direct microscopic examination and culture.

2.2 Laboratory methods

To test for the presence of invasive candidiasis, axilla and rectum swabs were inoculated. For direct examination, 10% KOH were applied, and the sample were evaluated under a microscope with 100 and 400 times of magnification. For culture, swab sample were inoculated in Sabouraud's dextrose agar plate. The plates were sealed and incubated at room temperature, and then were identified 24-72 hours later.

2.3 Definitions

Independent variables were the use of broad-spectrum antibiotics for more than 13 days, nutrition parenteral for more than 7 days, catheter venous central for more than 10 days, major surgery, digestive surgery, mechanical ventilation for more than 72 hours, haemodialysis, corticosteroid, septic, SOFA score more than 5, PCT < 1 ng/mL, CRP < 75 mg/L, neutrophil < 1000 cell/mm3, leucocyte < 10.000 cell/mm3, ESR > 15 mm, body temperature < 36°C or > 38°C. Colonization was defined as presence of increase Candida species sample and morphology was defined as presence of change from yeast to hyphae or pseudo hyphae in axilla and rectal swabs.

2.4 Statistical methods

To create predictive model, variables from univariate were selected based on the value of OR> 1 and p-value < 0.05. Univariates analysis was used for determining mean, median, modus, and proportion. Bivariate analysis was used Kaplan Meier and Log rank for independent variable related time. This variable was included in the global test, which means that the variable was fulfill the proportional hazard assumption. The backward method was used for selecting risk factors. Multivariate analyses used Cox regression. Statistical analysis was performed using STATA version 15 for Mac.

2.5 Result

One hundred forty-two subjects have been recruited in this study. Seventeen subjects were excluded in this

analysis cause include to exclusion criteria. During the study period, Candida morphology change and colonization increase in axilla and rectal swabs was detected in twenty-six subjects (22,6%). We compared the results of the first day of examination with the fifth day and the fifth day with the ninth day. The subject is regarded as the dependent variable if there is a change in the morphology of candida and an increase in colonization. The characteristic of demographic, clinical, laboratory test was shown in Table 1-3.

Bivariate analysis shows how strong each independent variable affects dependent variable. A patient's with older age more than sixty years old (HR 1.57; 95% CI .722-3.53), parenteral nutrition more than seven days (HR 1.49; 95% CI .66-3.34), major surgery (HR 1.2; 95% CI .705-2.32), digestive surgery (HR 1.43; 95% CI .62-3.30), Procalcitonin day 1 (HR 1.31; 95% CI 0.59-2.90), Procalcitonin day 5 (HR 2.01;95% CI .89-4.51), Procalcitonin day 9 (HR 1.23; 95% CI 0.47-3.22), CRP day 5 (HR 1.50; 95% CI .634-3.58), platelet day 1 (HR 1.37; 95% CI .55-3.41), platelet day t (HR 1.27; 95% CI .438-3.69), leukocyte day 1 (HR 1.02; 95% CI .41-2.55), leukocyte day 5 (HR 1.31; 95% CI .573-3.03), ESR day 5 (HR 2.69; 95%CI .364-19.86), and body temperature day 5 (HR 1.1; 95% CI .33-3.66) were significantly associated with higher hazard ratio of being Candida morphology change and colonization increase in axilla and rectal swabs.

Table 1. Demographic and clinical characteristic proportions and the Crude Hazard Ratios (95% CI) for Candida morphology change and colonization increase

Characteristics	**Total N=115 (100%)**	**Crude HR (95% CI)**	**p-value**
Age			
≤ 60 years	87 (75,6%)	1.57 (.702-3.53)	0.269
> 60 years	28 (24,4%)		
Gender			
Male	68 (59,1%)	.861 (.390-1.89)	0.712
Female	47 (40,9%)		
Diabetes mellitus			
Absent	88 (76,5%)	.832 (.313-2.20)	0.713
Present	29 (23,5%)		
Renal failure			
Absent	79 (68,7%)	.904 (.393-2.08)	0.814
Present	36 (31,3%)		
Septic			
Absent	42 (36,5%)	.361 (.164-.796)	0.012
Present	73 (63,5%)		
SOFA score			
≤ 5	71 (61,7%)	.769 (.342-1.72)	0.526
> 5	44 (38,3%)		
Body temperature Day 1			
36°C - 38°C	102 (88,7%)	.298 (.040-2.20)	0.235
< 36 °C or > 38°C	13 (11,3%)		
Body temperature Day 5			
36°C - 38°C	79 (68,7)	1.10 (.330-3.66)	0.875
< 36 °C or > 38°C	10 (8,7)		
Body temperature Day 9			
36°C - 38°C	82 (93,2%)	.763 (.102-5.70)	0.793

< 36 °C or > 38°C	6 (6,8%)

Table 2. Clinical managements profile proportions and the Crude Hazard Ratios (95%CI) for Candida morphology change and colonization increase

Characteristics	**Total N=115 (100%)**	**Crude HR (95% CI)**	**p-value**
Broad-spectrum antibiotics			
Absent	89 (77,4%)	.936 (.375-2.33)	0.887
Present	26 (22,6%)		
Parenteral nutrition			
Absent	86 (74,8%)	1.49 (.665-3.34)	0.332
Present	29 (25,2%)		
Central venous catheter			
< 10 days	52 (45,2%)	.835 (.385-1.80)	0.648
≥ 10 days	63 (54,8%)		
Major surgery			
Absent	87 (75,6%)	1.28 (.705-2.32)	0.415
Present	28 (24,4%)		
Digestive surgery			
Absent	89 (77,4%)	1.43 (.623-3.30)	0.395
Present	26 (22,6%)		
Mechanical ventilation			
≤ 5 days	37 (32,2%)	.894 (.413-1.93)	0.778
> 5 days	78 (67,8%)		
Haemodialysis			
Absent	79 (68,7%)	.904 (.393-2.08)	0.814
Present	36 (31,3%)		
Corticosteroids			
Absent	94 (81,7%)	.513 (.153-1.71)	0.278
Present	21 (18,3%)		

Table 3. Laboratory tests profile proportions and the Crude Hazard Ratios (95%CI) for Candida morphology change and colonization increase

Characteristics	**Total N=115 (100%)**	**Crude HR (95% CI)**	**p-value**
Procalcitonin Day 1			
> 1 ng/dl	75 (65,2%)	1.31 (.596-2.90)	0.496
≤ 1 ng/dl	40 (34,8%)		
Procalcitonin Day 5			
> 1 ng/dl	56 (48,7%)	2.01 (.895-4.51)	0.091
≤ 1 ng/dl	59 (51,3%)		
Procalcitonin Day 9			
> 1 ng/dl	30 (34%)	1.23 (.476-3.22)	0.661
≤ 1 ng/dl	58 (66%)		
CRPDay 1			
< 75 mg/l	27 (23,5%)	0.775 (.325-1.84)	0.566

≥ 75 mg/l	88 (76,5%)		
CRPDay 5			
< 75 mg/l	41 (35,6%)	1.50 (.634-3.58)	0.352
≥ 75 mg/l	74 (64,4%)		
CRP Day 9			
< 75 mg/l	40 (45,5%)	.963 (.391-2.37)	0.936
≥ 75 mg/l	48 (54,5%)		
Platelets Day 1			
>100.000cell/mm^3	95 (82,6%)	1.37 (.550-3.41)	0.498
≤100.000cell/mm^3	20 (17,4%)		
Platelets Day 5			
>100.000cell/mm^3	99 (86,1%)	1.27 (.437-3.69)	0.658
≤100.000cell/mm^3	16 (13,9%		
Platelets Day 9			
>100.000cell/mm^3	80 (90,9%)	.576 (.077-4.32)	0.592
≤100.000cell/mm^3	8 (9%)		
Leukocyte Day 1			
≥ 10.000 cell/mm^3	90 (78,3%)	1.02 (.412-2.55)	0.953
< 10.000 cell/mm^3	25 (21,7%)		
Leukocyte Day 5			
≥ 10.000 cell/mm^3	84 (73%)	1.31 (.573-3.03)	0.515
< 10.000 cell/mm^3	31 (27%)		
Leukocyte Day 9			
≥ 10.000 cell/mm^3	71 (81,8%)	0.49 (.113-2.13)	0.344
< 10.000 cell/mm^3	16 (18,2%)		
ESR Day 1			
≤ 15 mm	9 (7,8%)	.315 (.118-.837)	0.021
> 15 mm	106 (92,2%)		
ESR Day 5			
≤ 15 mm	11 (9,6%)	2.69 (.364-19.8)	0.332
> 15 mm	104 (90,4%)		
ESR Day 9			
≤ 15 mm	9 (10,3%)	.955 (.220-4.13)	0.952
> 15 mm	78 (89,7%)		

Abbreviation: CRP: C-Reactive Protein, ESR: Erythrocyte Sedimentation Rate

The Proportional Hazard assumption is fulfilled by all of the variables below, according to the results of the global test on all variables, which indicate a p value > 0.05, or 0.7555 shown in Table 4.

Table 4. Cox Proportional Hazard Assumption multivariate analysis

Characteristic	**Correlation (Chi^2)**	***p-value***
Parenteral nutrition	1.13	0.2888
Central venous catheter	0.05	0.8283
SOFA score	1.68	0.1947
CRP day 1	0.85	0.3554
Corticosteroid	0.82	0.3644

Broad-spectrum antibiotic	0.07	0.7891
CRP day 5	0.00	0.9896
Leukocyte day 1	0.04	0.8431
Leukocyte day 5	5.13	0.0235
Leukocyte day 9	3.13	0.0767
Body temperature day 5	2.83	0.0926
Body temperature day 9	0.00	1.0000
Elder age	3.75	0.0529
Female	1.43	0.2326
Renal failure	0.60	0.4387
Global test	13.09	0.5956

Parenteral nutrition, leukocyte day 1, elder age and female have a hazard ratio more than one, which it means all risk was associated with Candida morphology change and colonization increase in axilla and rectal swabs shown in Table 5.

Table 5. Multivariate hazard ratio

Characteristic	***Hazard Ratio***	**95% CI**		***p-value***
		Min	**Max**	
Parenteral nutrition	2.739	.961	7.806	.059
Leukocyte day 1	3.065	1.088	8.633	.034
Elder age	2.553	.860	7.577	.091
Female	1.636	.537	4.984	.387

2.6. Discussion

ICU patients are well-known to carry a greater risk of developing fungal infections. Candida colonization is one of such crucial risk factors. Candida species typically colonize the uncircumcised males' foreskin, groin, lower female genital tract, intertriginous areas (such as the armpits and groin), and skin. The respiratory tract is also the first to be colonized, therefore the skin and gastrointestinal tract.[6]Since invasive candidiasis is preceded by colonization of mucosal surfaces with the infecting strain [7-9] and since colonization is an independent risk factor for invasive candidiasis [2,7,9], it is logical that it should be incorporated into the predictive models.[2-5]

This study determined that the collection of samples from two sites (axilla and rectum) was practical and feasible but not necessary for routine procedures. Patients were observed during a nine-day periodsince the incubation of fungal culture would be detected between seven and fourteen days.[10]Based on the findings of this study, parenteral nutrition, leukocyte day 1, old age, and female gender have strength relationship with Candida morphology changes and colonization increase in axilla and rectal swab. Parenteral nutrition was also identified as a risk factor of Candida infection. Several elements of parenteral nutrition were considered to increase the risk of bacteria development. Lipid emulsion containing solutions encouraged the development of microorganisms, and the probability increases when they were not removed or changed for a duration longer than 24 hours. Moreover, the pH of the solution may raise the risk of colonization and infection.[11]Due to several circumstances, including an increased prevalence of comorbidities, aging-related physiological changes, concomitant drug usage, and a high colonization rate, the elderly are particularly vulnerable to Candida infection.[12]

Clinicians may implement the colonization evidence to assist empirical treatment decisions through the use of this method, which considers Candida colonization as a diagnostic test and determines significant

diagnostic test parameters. However, Candida morphology changes and colonization increase present high negative predictive value to avoid unnecessary empirical antifungal use in critically ill patients. Nonetheless, the positive predictive value of using Candida colonization was low, suggesting that empirical antifungal coverage is presumably not warranted.[13]

3. Conclusion

In conclusion**,** parenteral nutrition, leukocyte day 1, old age and female have a hazard ratio more than one, which it means all risk was associated with Candida morphology change and colonization increase in axilla and rectal swabs. Further research is needed to determine the likelihood Candida morphology change and colonization increase in axilla and rectal swabs, such as system scoring. It will help clinician to know how to start antifungal administration.

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